



Short communication: Fractional milking distribution of immunoglobulin G and other constituents in colostrum

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ABSTRACT

The provision of quality colostrum with a high concentration of immunoglobulins is critical for newborn calf health. Because first colostrum may be low in overall concentration to effectively reduce the risk of newborn infections, we tested equivalent milking fractions of colostrum for possible IgG differences. The objective of this study was to determine if the fractional composition of colostrum changes during the course of milking with a focus on immunoglobulins. Twenty-four Holstein and Simmental cows were milked (first colostrum) within 4 h after calving. The colostrum of 1 gland per animal was assembled into 4 percentage fractions over the course of milking: 0 to 25%, 25 to 50%, 50 to 75%, and 75 to 100%. The IgG concentration among the various fractions did not change in any significant pattern. Concentration of protein, casein, lactose and somatic cell count remained the same or exhibited only minor changes during the course of fractional milking colostrum. We determined that no benefit exists in feeding any particular fraction of colostrum to the newborn.

Key words: colostrum, immunoglobulin G, fraction

Short Communication

Sufficient and timely absorption of colostrum immunoglobulins is vital for newborn calves (Morrill et al., 2012). In addition, other colostrum nutrients (fat, protein, and vitamins) and factors with regulative function (cytokines, growth factors, enzymes, and hormones) are important in the development of the immune defense systems (leukocytes and lactoferrin). Colostrum IgG1 supplies the initial immune defense that the calf requires for the first weeks of its life because the epitheliochorial placenta of cattle does not allow the passage of immunoglobulins during pregnancy (Chucrí et al., 2010). If the intake of antibodies from the colostrum

is insufficient or absent, a significantly higher risk of morbidity or mortality exists (McGuire et al., 1976; Donovan et al., 1986; Rea et al., 1996). Because the calf's limited ability to consume a high volume at birth and IgG1 absorption from the intestine decreases significantly during the first 24 h (Matte et al., 1982), sufficient IgG concentration in the consumed colostrum of first milking is a prerequisite for the protective passive immune defense of the newborn calf.

At the very end of pregnancy, the mammary gland begins to synthesize mature milk components, but this beginning time period likely is highly variable among animals. One of these mature components is lactose, the principal osmole of mature milk. The prepartum appearance of lactose causes movement of water into colostrum that dilutes the concentration of IgG both prepartum and especially postpartum, when substantial delay of first colostrum milking occurs (Morin et al., 2010). Because the milk volume of first colostrum after calving is negatively correlated with immunoglobulin concentration (Pritchett et al., 1991), the concentration in the first colostrum of dairy cows is frequently insufficient for optimum calf intake.

Stott et al. (1981) examined the possibility that first 100 mL of first milked colostrum from 12 Holstein cows may contain a more concentrated IgG content than that of subsequent fractions. Although that study showed no difference in the collected fractions, the IgG concentration difference between the fractions was large in some animals and the use of 12 animals may have concealed the identification of significant differences. Hostetler et al. (2003) showed that a hand-stripped post-milking-machine sample of colostrum exhibited lower IgG concentration ($P < 0.05$) than a hand premilking sample (10 mL) as well as the remaining milking machine fraction. Studies of colostrum fractionation from d 2 (Ontsouka et al., 2003) and of mature milk (Ontsouka et al., 2003; Bruckmaier et al., 2004; Sarikaya et al., 2005; Tančin et al., 2007) found that milk composition changes during the course of milking, which corresponds to a continuous character of milk ejection during milk removal (Bruckmaier et al., 1994;

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Bruckmaier and Blum, 1998). Thus, the concept that colostrum composition could also be different during the first milking remained unclear.

Previous studies on immunoglobulin concentration in colostrum have been largely studied by immunodiffusion methods. Based upon previous colostrum fractionation studies, we hypothesized that a specific portion of first-milked colostrum obtained during the course of milking would contain higher concentrations of immunoglobulins. The objective of this study was to examine the fractional composition of the first colostrum during the course of milking. If portions of first-milked colostrum differed in IgG concentration, the possibility of feeding the newborn calf specific colostrum fractions with the highest concentration of immunoglobulin could improve calf health.

Fourteen cows of the Swiss Federal Station in Posieux, Switzerland (Agroscope Liebefeld-Posieux ALP), plus 10 Simmental cows from a family-owned farm in Matzendorf (Switzerland) were used for this study. All animals were kept in a loose housing barn and during calving they were kept in a separate calving facility.

To establish the udder health status of the 14 cows in Posieux, first-colostrum samples were aseptically collected and bacteriologically tested (Institute of Veterinary Bacteriology, Bern, Switzerland). Thirteen of the bacteriological tests showed no udder pathogenic microbes, and only 1 cow showed a low count of *Streptococcus uberis* in her milk. We concluded that this result was most likely due to contamination during sampling because the low SCC before drying off continued into the colostrum stage. To ensure the absence of mastitis of the cows from Matzendorf, the SCC was required to be <100,000 per mL in the last month before drying off and at the first 2 analyses within 2 mo after calving. Of the experimental animals, 7 cows each were in the second and third lactation, 2 in the fourth, 4 in the fifth, 3 in the sixth, and 1 in the seventh lactation. Cows calved without complications with no periparturient clinical disorders.

All 24 cows were milked within the first 4 h after calving (colostrum of the first milking). All colostrum samples were collected by the same individual. Milk from 1 udder quarter was expeditiously milked by hand to collect 50 mL into serially numbered containers. The total weight of the colostrum from this gland was recorded. The colostrum fractions were then distributed into 4 homogeneous samples of the same percentage weight: 0 to 25% of removed milk = first fraction (**F25**), 25 to 50% = second fraction (**F50**), 50 to 75% = third fraction (**F75**), and 75 to 100% = fourth fraction (**F100**). All samples were frozen (-20°C) until analysis.

In most cases, the right rear gland was used for fractionized milking. However, in 8 cases, the front right

gland was used because the rear teat was leaking milk. Nevertheless, the same gland was always used for colostrum fractionation.

The IgG concentration was determined using the bovine IgG ELISA quantitation set (catalog no. E10-118) of Bethyl Laboratories Inc. (Montgomery, TX) at the laboratory of the Veterinary Physiology group of the University Bern (Vetsuisse Faculty, Posieux, Switzerland). Fractions of colostrum for this assay used a 2×10^5 , 4×10^5 , 8×10^5 , and 1.6×10^6 dilution factor, which were obtained in sequential 1:10 dilution steps, with the final four 1:2 dilutions conducted within the ELISA plate. These concentrations were used to achieve a coefficient of variation of <12% of the measured within-plate samples. Samples that exceeded the set coefficient of variation were assayed again with adjusted dilutions. The ELISA was carried out according to the guidelines of the manufacturer. In addition to the manufacturer blocking solution we added diluted gelatin [5% in Tris-buffered saline (TBS)-Tween] from coldwater fish skin (Sigma-Aldrich, St Louis, MO; ~45% in H_2O ; G7765) to reduce nonspecific binding. Furthermore, each fraction was analyzed for protein, casein, fat, lactose, and SCC at the laboratory of the Holstein Association of Switzerland (Grangeneuve, Switzerland; CombiFoss 6000; Gerber Instruments AG, Effretikon, Switzerland) after fractions were diluted 1:2 with 0.9% NaCl solution.

The IgG ELISA sample dilution results had a coefficient of variation of <12%. Data were analyzed with PROC MIXED of SAS (SAS Institute, 2007). Differences between fractions were tested for significance ($P < 0.05$) using fraction as fixed effect. Influences of parity, yield of quarter, or yield of udder half, on colostrum IgG concentration were tested in preliminary models. Because none of these factors had a significant effect, they were removed from the final model. Statistical significance between fractions was tested by Bonferroni *t*-test. Because SCC could not be assumed to be normally distributed, the SCC values were converted to logarithmic value for statistical analysis. Data are presented as means \pm standard error.

The mean milk yield (**MY**) of colostrum per gland was $1,391 \pm 305$ g. Seven of the cows yielded <1,000 g per gland, 13 yielded between 1,000 and 2000 g, and 4 cows more than 2,000 g per gland.

Figure 1 shows the IgG concentration that occurred in the 4 equal fractions of the first milking of colostrum. Only a few animals had different concentrations of IgG between fractions (F25 to F100) and the overall result was no difference in fraction concentration. This can also be observed in the results shown in Table 1. In addition to IgG concentration, no difference existed in protein, casein, or lactose concentration, or SCC be-

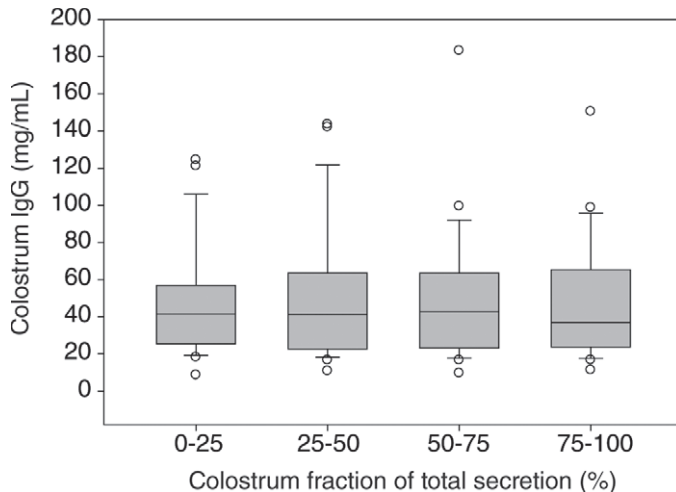


Figure 1. Immunoglobulin G concentration in the 4 fractions of first-milked colostrum. Data are from 1 quarter from each cow ($n = 24$). The line in the box is the median, with close and far boundary representing the 25th and 75th percentile. Outliers are shown outside the box.

tween the 4 fractions during the course of first milking after calving. Analysis of parity (second lactation vs. more than 2 lactations), MY per teat ($MY < 1,000$ g vs. $1,000 \text{ g} \leq MY \leq 2,000$ g vs. $MY > 2,000$ g), and udder half (front vs. rear) showed no significant effect on constituents during the course of milking (data not shown).

Our results obtained by an IgG ELISA show no significant change in IgG concentration during the fractional course of milking. Stott et al. (1981) reported that the first 100 mL of colostrum milking was not different in concentration than the remaining colostrum volume, using the immunodiffusion method of Fahey and McKelvey (1965). Although they showed no significant differences between the milking fractions, their data indicated large concentration differences, with some animals showing lower concentration in the first 100 mL fraction. Hostetler et al. (2003) showed no difference between a precolostrum hand-stripped sample and machine milking fraction, but showed a decrease in

concentration in a hand-stripped sample after machine milking. Ontsouka et al. (2003) examined fractionized milk of IgG composition with d-2 colostrum (third milking), also by an immunodiffusion technique. Their samples were the first 100 mL of cisternal colostrum/milk. They found IgG concentration differences, but these were not significant. Therefore, based upon previous findings and our current data, we reject our hypothesis of a colostrum fraction that exhibits a higher concentration of IgG.

We found that fat content is the only colostrum constituent to show changes during the course of fractional milking in the current study. This increase has been reported in many studies (Ontsouka et al., 2003; Bruckmaier et al., 2004; Sarikaya et al., 2005; Tančin et al., 2007). It is likely that the fat droplets, which are delimited by lipoprotein membranes, are held back by capillary forces in the alveoli and through adhesive forces on the udder epithelium during the process of milking. Because cows release oxytocin during calving, it can be assumed that milk ejection occurs, which would cause blending of low-fat cisternal milk with some small amount of the high-fat alveolar milk. Tančin et al. (2007) showed that the increase in fat concentration during milking proceeded more smoothly when premilking udder stimulation was applied.

The SCC in our study remained constant across the milking fractions, whereas lactose content decreased slightly from F25 to F100 fractions (Table 1). Other studies found a similar lactose and protein content (Ontsouka et al., 2003; Bruckmaier et al., 2004; Sarikaya et al., 2005). The reasons for the decrease over the course of milking of both protein and lactose are unclear, but it is conceivable that contents of water-soluble milk constituents decrease when fat content rises towards the end of milking. The decrease in cisternal milk described in the above study could at least partly be ascribed to the fact that the residual milk, which contains lower protein and lactose concentrations, is mixed with the cisternal milk of the next milking and, thus, reduces its content.

Table 1. Colostrum from the first milking ($n = 24$), with fractions obtained from 1 quarter

Component	Fraction ¹			
	F25	F50	F75	F100
IgG (g/L)	44.5 ± 5.2	49.9 ± 7.3	49.8 ± 7.5	47.2 ± 6.7
Protein (g/100 g)	17.1 ± 0.8	17.0 ± 0.8	16.9 ± 0.8	16.7 ± 0.7
Casein (g/100 g)	11.4 ± 0.6	11.2 ± 0.6	11.2 ± 0.6	11.0 ± 0.6
Fat (g/100 g)	3.8 ± 0.5	4.0 ± 0.5	4.7 ± 0.6	5.7 ± 0.7
Lactose (g/100 g)	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1
SCC (log cells/mL)	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1

¹F25 = 0 to 25% of removed milk (in percentage of weight; first fraction); F50 = 25 to 50% (second fraction); F75 = 50 to 75% (third fraction); F100 = 75 to 100% (fourth fraction).

The SCC in our study remained constant across the milking fractions. Cell types are reported to vary in their proportion in different milk fractions; macrophages represent the predominant cell fraction in cisternal milk, whereas PMNL make up the predominant cell fraction in alveolar milk (Sarıkaya et al., 2005). Bruckmaier et al. (2004) proposed that milk immune cells, because of their polar membrane, are subject to the same adhesive forces as are fat cells or that they are partially attached to the milk fat fraction.

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REFERENCES

- Bruckmaier, R. M., and J. W. Blum. 1998. Oxytocin release and milk removal in ruminants. *J. Dairy Sci.* 81:939–949.
- Bruckmaier, R. M., C. E. Ontsouka, and J. W. Blum. 2004. Fractionized milk composition in dairy cows with subclinical mastitis. *Vet. Med. – Czech* 49:283–290.
- Bruckmaier, R. M., D. Schams, and J. W. Blum. 1994. Continuously elevated concentrations of oxytocin during milking are necessary for complete milk removal in dairy cows. *J. Dairy Res.* 61:323–334.
- Chucrí, T. M., J. M. Monteiro, A. R. Lima, M. L. B. Salvadori, J. R. Kfoury Jr., and M. A. Miglino. 2010. A review of immune transfer by the placenta. *J. Reprod. Immunol.* 87:14–20.
- Donovan, G. A., L. Badinga, R. J. Collier, C. J. Wilcox, and R. K. Braun. 1986. Factors influencing passive transfer in dairy calves. *J. Dairy Sci.* 69:754–759.
- Fahey, J. L., and E. M. McKelvey. 1965. Quantitative determination of serum immunoglobulins in antibody-agar plates. *J. Immunol.* 94:84–90.
- Hostetler, D., V. L. Douglas, J. Tyler, J. Holle, and B. Steevens. 2003. Immunoglobulin G concentrations in temporal fractions of first milking colostrum in dairy cows. *Int. J. Appl. Res. Vet. Med.* 1:168–171.
- Matte, J. J., C. L. Girard, J. R. Seoane, and G. J. Brisson. 1982. Absorption of colostral immunoglobulin G in the newborn calf. *J. Dairy Sci.* 65:1765–1770.
- McGuire, T. C., N. E. Pfeiffer, J. M. Weikel, and R. C. Bartsch. 1976. Failure of colostral immunoglobulin transfer in calves dying from infectious disease. *J. Am. Vet. Med. Assoc.* 169:713–718.
- Morin, D. E., S. V. Nelson, E. D. Reid, D. W. Nagy, G. E. Dahl, and P. D. Constable. 2010. Effect of colostral volume, interval between calving and first milking, and photoperiod on colostral IgG concentrations in dairy cows. *J. Am. Vet. Med. Assoc.* 237:420–428.
- Morrill, K. M., E. Conrad, A. Lago, J. Campbell, J. Quigley, and H. Tyler. 2012. Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States. *J. Dairy Sci.* 95:3997–4005.
- Ontsouka, C. E., R. M. Bruckmaier, and J. W. Blum. 2003. Fractionized milk composition during removal of colostrum and mature milk. *J. Dairy Sci.* 86:2005–2011.
- Pritchett, L. C., C. C. Gay, T. E. Besser, and D. D. Hancock. 1991. Management and production factors influencing immunoglobulin G₁ concentration in colostrum from Holstein cows. *J. Dairy Sci.* 74:2336–2341.
- Rea, D. E., J. W. Tyler, D. D. Hancock, T. E. Besser, L. Wilson, D. S. Krytenberg, and S. G. Sanders. 1996. Prediction of calf mortality by use of tests for passive transfer of colostral immunoglobulin. *J. Am. Vet. Med. Assoc.* 208:2047–2049.
- Sarıkaya, H., C. Werner-Misof, M. Atzkern, and R. M. Bruckmaier. 2005. Distribution of leucocyte populations, and milk composition, in milk fractions of healthy quarters in dairy cows. *J. Dairy Res.* 72:486–492.
- SAS Institute. 2007. SAS/STAT Software. Version 9. SAS Institute Inc., Cary, NC.
- Stott, G. H., W. A. Fleenor, and W. C. Kleese. 1981. Colostral immunoglobulin concentration in two fractions of first milking postpartum and five additional milkings. *J. Dairy Sci.* 64:459–465.
- Tančin, V., M. Uhrinčat, L. Mačuhová, and R. M. Bruckmaier. 2007. Effect of pre-stimulation on milk flow pattern and distribution of milk constituents at a quarter level. *Czech J. Anim. Sci.* 52:117–121.